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(54) Title: NOVEL IMMUNOSUPPRESSIVE COMPOUNDS

(57) Abstract

This invention relates to a novel class of immunosuppressive compounds having an affinity for the FK-506 binding protein (FKBP). Once bound to this protein, the immunosuppressive compounds inhibit the prolyl peptidyl cis-trans isomerase (rotamase) activity of the FKBP and inhibit T cell activation. As such, the compounds of this invention can be used as immunosuppressive drugs to prevent or significantly reduce graft rejection in bone marrow and organ transplantations and for use in the treatment of a wide variety of autoimmune diseases in humans and other mammals.

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NOVEL IMMUNOSUPPRESSIVE COMPOUNDS

Background of the Invention

Post operative graft rejections are a major complication affecting the success of bone marrow and organ transplantations. However, through the use of immunosuppressive drug therapy, graft rejection in organ transplantation can be significantly reduced.

A wide variety of diseases can be characterized as "autoimmune diseases". Such diseases are similar to graft rejection, except that the rejection is of self tissue. Immunosuppressive therapy can also be of use in preventing this inappropriate self rejection.

One widely accepted immunosuppressant for the prevention of graft rejection is cyclosporin A (CsA). It is a natural product of fungal metabolism and has been demonstrated to have potent immunosuppressive activity in clinical organ transplantations. Calne, R.Y. et al., Br. Med. J. 282:934-936 (1981); White, D.J.C. Drugs 24:322-334 (1982). Although CsA is widely used in immunosuppressant therapy, its usage (particularly in high dosage) is often accompanied by side effects which include nephrotoxicity, hepatotoxicity and other central nervous system disorders.

The following diseases have been treated with cyclosporin A with positive results, confirming the importance of the autoimmune component in these diseases and their effective treatment with compounds working by selective T-cell immune suppression similar to cyclosporin A.

1) Ophthalmology: Uveitis, Behcet's disease and Grave's ophthalmopathy.

Weetman, A.P. et al., Lancet 486-489 (1982). Grave's opthalmopathy.

Nussenblatt, R.B. et al., Lancet 235-238 (1983). Uveitis.

French-Constant, C. et al., Lancet 454 (1983). Behcet's disease.

Sanders, M. et al., Lancet 454-455 (1983). Behcet's disease.

Note: Cyclosporin A is currently approved in Japan for the treatment of Behcet's disease, the first autoimmune disease indication for this compound.

2) Dermatology: Various autoimmune skin diseases including psoriasis.

Zabel, P. et al., Lancet 343 (1984). Acute dermatomyositis.

van Joost, T. et al., Arch. Dermatol. 123:166-167 (1987). Atopic skin disease.

Appleboom, T. et al., Amer. J. Med. 82:866-867 (1987). Scleroderma.

Logan, R.A. and R.D.R. Camo, J. Roy. Soc. Med. 81:417-418 (1988). Eczema.

Griffiths, C.E.M. et al., Brit. Med. J. 293:731-732 (1986). Psoriasis.

Ellis, C.N. et al., J. Amer. Med. Assoc. 256:3110-3116 (1986). Psoriasis.

3) Hematology: Various diseases including anemia.

Toetterman, T.H. et al., Lancet, 693 (1984). Pure red cell aplasia (PRCA).

Stryckmans, P.A. et al., New Engl. J. Med. 310:655-656 (1984). Aplastic anemia.

Gluckman, E. et al., Bone Marrow Transplant 3 Suppl. 1, 241 (1988). Aplastic anemia.

4) Gastroenterology/Hepatology: Primary cirrhosis, autoimmune hepatitis, ulcerative colitis, Crohn's disease and other gastrointestinal autoimmune diseases.

Wiesner, R.H. et al., Hepatology 7:1025, Abst. #9, (1987). Primary biliary cirrhosis.

Hyams, J.S. et al., Gastroenterology 93:890-893 (1987). Autoimmune hepatitis.

Allison, M.C. et al., Lancet, 902-903 (1984). Crohn's disease.

Brynskov, J. et al., Gastroenterology 92:1330 (1987). Crohn's disease.

Porro, G.B. et al., Ital. J. Gastroenterol. 19:40-41 (1987). Ulcerative colitis.

5) Neurology: Amyotrophic lateral sclerosis (ALS, "Lou Gehrig's disease"), myasthenia gravis and multiple sclerosis.

Appel, S.H. et al., Arch. Neurol. 45:381-386 (1988). ALS.

Tindall, R.S.A. et al., New Engl. J. Med. 316:719-724 (1987). Myasthenia gravis.

Ann. Neurol. 24, No. 1, p. 169,m Abstract P174 (1988). Multiple sclerosis.

Dommasch, D. et al., Neurology 38 Suppl. 2, 28-29 (1988). Multiple sclerosis.

6) Nephrotic Syndrome: Nephrotic syndrome, membrano-proliferative glomerulonephritis (MPGN) and related diseases.

Watzon, A.R. et al., Clin. Nephrol. 25:273-274 (1986). Nephrotic syndrome.

Tejani, A. et al., Kidney Int. 33:729-734 (1988). Nephrotic syndrome.

Meyrier, A. et al., Transplant Proc. 20, Suppl. 4 (Book III), 259-261 (1988). Nephrotic syndrome.
LaGrue, G. et al., Nephron. 44:382-382 (1986). MPGN.

7) Rheumatoid Arthritis (RA)
Harper, J.I. et al., Lancet 981-982 (1984). RA
Van Rijthoven, A.W. et al., Ann. Rheum. Dis. 45:726-731 (1986). RA.
Dougados, M. et al., Ann. Rheum. Dis. 47:127-133 (1988). RA.

8) Insulin-Dependent Diabetes Mellitus (IDDM)
Stiller, C.R. et al., Science 223:1362-1367 (1984). IDDM.
Assan, R. et al., Lancet, 67-71 (1985). IDDM.
Bougneres, P.F. et al., New Engl. J. Med. 318:663-670 (1988). IDDM.
Diabetes 37:1574-1582 (1988). IDDM.

Many veterinary diseases are also characterized as autoimmune diseases. Autoimmune diseases such as those listed above have been observed in mammals. Papa, F.O. et al., Equine Vet. J. 22:145-146 (1990) infertility of autoimmune origin in the stallion; Gorman, N.T. and L.L. Werner, Brit. Vet. J. 142:403-410, 491-497 and 498-505 (1986) immune mediated diseases of cats and dogs; George, L.W. and S.L. White, Vet. Clin. North Amer. 6:203-213 (1984) autoimmune skin diseases in large mammals; Bennett, D., In. Pract. 6:74-86 (1984) autoimmune diseases in dogs; Halliwell, R.E., J. Amer. Vet. Assoc. 181:1088-1096 (1982) autoimmune diseases in domesticated animals.

The mechanism by which CsA causes immunosuppression has been established. In vitro, CsA inhibits the release of lymphokines, such as interleukin 2 (IL-2) [Bunjes, D. et al., Eur. J. Immunol. 11:657-661 (1981)] and prevents clonal expansion of helper and cytotoxic T cells [Larsson, E. J. Immunol. 124:2828-2833 (1980)]. CsA has been shown to bind the cytosolic protein, cyclophilin, and inhibit the prolyl-peptidyl cis-trans isomerase (PPIase) activity of that protein. Fischer, G. et al., Nature 337:476-478 (1989); Takahashi, N. et al., Nature 337:473-475 (1989). The PPIases may mediate T cell activation by catalyzing the rotomerization of peptide bonds of prolyl residues.

Recently, a second natural product isolated from Streptomyces, referred to as FK-506, has been demonstrated to be a potent immunosuppresive agent. Tanaka, H. et al., J. Am. Chem. Soc. 109:5031-5033 (1987). FK-506 inhibits IL-2 production, inhibits mixed lymphocyte culture response and inhibits cytotoxic T-cell generation in vitro at 100 times lower concentration than cyclosporin A. Kino, T. et al., J. Antibiot. 15:1256-1265 (1987). FK-506 also inhibits PPIase activity, but is structurally different from CsA and binds to a binding protein (FKBP) distinct from cyclophilin. Harding, M.W. et al., Nature 341:758-760 (1989); Siekierka, J.J., Nature 341:755-757 (1989).

Summary of the Invention

This invention relates to a novel class of immunosuppressive compounds having an affinity for the FK-506 binding protein (FKBP). Once bound to this protein, the immunosuppressive compounds inhibit the prolyl peptidyl

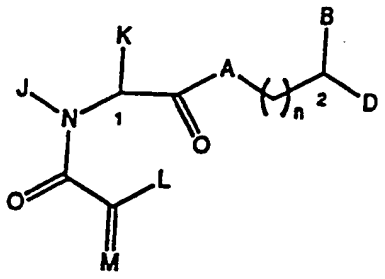
cis-trans isomerase (rotamase) activity of the FKBP and lead to inhibition of T cell activation. The compounds of this invention can be used as immunosuppressive drugs to prevent or significantly reduce graft rejection in bone marrow and organ transplantations and in the treatment of autoimmune disease in humans and other mammals.

Brief Description of the Drawings

Figures 1A-1I illustrate some preferred compounds of this invention. The synthesis of each of the preferred compounds is described in detail in the Example section.

Detailed Description of the Invention

This invention relates to a novel class of immunosuppressive compounds represented by the formula I:



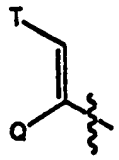
I

and pharmaceutically acceptable salts thereof,

wherein A is CH_2, oxygen, NH, or N-(C1-C4 alkyl);

wherein B and D are independently Ar, (C5-C7)-cycloalkyl substituted (C1-C6)-straight or branched alkyl or alkenyl, (C5-C7)-cycloalkenyl substituted (C1-C6)-straight or branched alkyl or alkenyl, or Ar substituted (C1-C6)-straight or branched

alkyl or alkenyl, wherein in each case, one or two carbon atoms of the straight or branched alkyl or alkenyl groups may be substituted with 1-2 heteroatoms selected from the group consisting of oxygen, sulfur, SO and SO_2 in chemically reasonable substitution patterns, or



wherein Q is hydrogen, (C1-C6)-straight or branched alkyl or (C1-C6)-straight or branched alkenyl;

wherein T is Ar or substituted 5-7 membered cycloalkyl with substituents at positions 3 and 4 which are independently selected from the group consisting of oxo, hydrogen, hydroxyl, O-(C1-C4)-alkyl and O-(C1-C4)-alkenyl;

wherein Ar is selected from the group consisting of phenyl, 1-naphthyl, 2-naphthyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, monocyclic and bicyclic heterocyclic ring systems with individual ring sizes being 5 or 6 which may contain in either or both rings a total of 1-4 heteroatoms independently selected from oxygen, nitrogen and sulfur; wherein Ar may contain one to three substituents which are independently selected from the group consisting of hydrogen, halogen, hydroxymethyl, hydroxyl, nitro, trifluoromethyl, trifluoromethoxy, (C1-C6)-straight or branched alkyl, (C1-C6)-straight or branched alkenyl, O-(C1-C4)-straight or branched alkyl, O-(C2-C4)-straight or branched alkenyl, O-benzyl, O-phenyl, 1,2-methylenedioxy, amino, carboxyl and phenyl;

wherein L is either hydrogen or U; M is either oxygen or CH-U, provided that if L is hydrogen, then M is CH-U or if M is oxygen then L is U;

wherein U is hydrogen, O-(C1-C4)-straight or branched alkyl, O-(C1-C4)-straight or branched alkenyl, (C1-C6)-straight or branched alkyl, (C1-C6)-straight or branched alkenyl, (C5-C7)-cycloalkyl, (C5-C7)-cycloalkenyl substituted with (C1-C4)-straight or branched alkyl or (C2-C4)-straight or branched alkenyl, [(C1-C4)-alkyl or (C2-C4)-alkenyl]-Ar or Ar (Ar as described above);

wherein J is hydrogen or C1 or C2 alkyl or benzyl; K is (C1-C4)-straight or branched alkyl, benzyl or cyclohexylmethyl; or wherein J and K may be taken together to form a 5-7 membered heterocyclic ring which may contain an O, S, SO or SO_2 substituent therein; and

wherein n is 0-3.

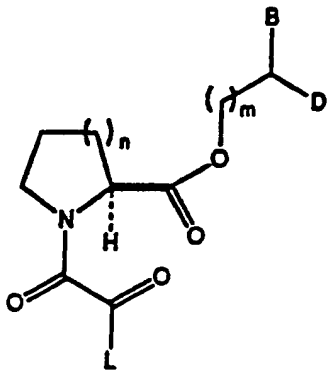
The stereochemistry at position 1 (Formula I) is (R) or (S), with (S) preferred. The stereochemistry at position 2 is (R) or (S).

The compounds of the present invention can be used in the form of salts derived from inorganic or organic acids and bases. Included among such acid salts are the following: acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, pamoate, pectinate, persulfate, 3-phenylpropionate, picrate, pivalate, propionate, succinate,

tartrate, thiocyanate, tosylate and undecanoate. Base salts include ammonium salts, alkali metal salts such as sodium and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, salt with organic bases such as dicyclohexylamine salts, N-methyl-D-glucamine, and salts with amino acids such as arginine, lysine, and so forth. Also, the basic nitrogen-containing groups can be quaternized with such agents as lower alkyl halides, such as methyl, ethyl, propyl, and butyl chloride, bromides and iodides; dialkyl sulfates like dimethyl, diethyl, dibutyl and diamyl sulfates, long chain halides such as decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides, aralkyl halides like benzyl and phenethyl bromides and others. Water or oil-soluble or dispersible products are thereby obtained.

Preferably, the compounds will have a molecular weight below about 750 atomic mass units (a.m.u.) and most preferably below about 500 a.m.u. Examples of compounds in which the J and K substituents are taken together to form a heterocyclic ring are shown in Table 1 and Figure 1.

TABLE 1: Compounds



No.	n	m	B	D	L
2	1	0	3-(2-Pyridyl)-propyl	3-Phenylpropyl	3,4,5-Trimeth-oxyphenyl
3	2	0	3-Phenylpropyl	3-Phenylpropyl	3,4,5-Trimeth-oxyphenyl
4	2	0	2-Phenoxy-phenyl	3-Phenylpropyl	3,4,5-Trimeth-oxyphenyl
5	2	0	Phenyl	2-Phenoxy-phenyl	3,4,5-Trimeth-oxyphenyl
6	2	0	Phenyl	3-Phenylpropyl	3,4,5-Trimeth-oxyphenyl
7	2	0	2-(3-pyridyl)-ethyl	3-Phenylpropyl	3,4,5-Trimeth-oxyphenyl
8	2	0	E-3-[trans-(4-Hydroxycyclo-hexyl)]-2-methyl-prop-2-enyl	3-Phenylpropyl	3,4,5-Trimeth-oxyphenyl
9	2	0	3-(3-Pyridyl-propyl	3-Phenylpropyl	3,4,5-Trimeth-oxyphenyl

Table 1 (continued)

No.	n	m	B	D	L
10	2	0	Benzyl	3-Phenylpropyl	3,4,5-Trimethoxyphenyl
11	2	0	Benzyl	3-(3-indolyl) propyl	3,4,5-Trimethoxyphenyl
12	2	0	2-Phenylethyl	3-Phenylpropyl	3,4,5-Trimethoxyphenyl
13	2	0	2-(4-Methoxyphenyl)ethyl	3-Phenylpropyl	3,4,5-Trimethoxyphenyl
14	2	0	2-(4-Methoxyphenyl)ethyl	3-Phenylpropyl	Phenyl
15	2	0	3-(N-benzimidazoly)propyl	3-Phenylpropyl	3,4,5-Trimethoxyphenyl
16	2	1	Benzyl	2-Phenylethyl	3,4,5-Trimeth-
17	2	0	3-(4-Methoxyphenyl)propyl	3-Phenylpropyl	3,4,5-Trimethoxyphenyl
18	2	0	3-(3-Pyridyl)-propyl	3-Phenylpropyl	Phenyl
19	2	0	3-(2-Pyridyl)-propyl	3-Phenylpropyl	Phenyl
20	2	0	3-(2-Pyridyl)-propyl	3-Phenylpropyl	3,4,5-Trimethoxyphenyl
21	2	0	3-(2-Pyridyl)-propyl	3-Phenylpropyl	tert-Butyl
22	2	0	3-(3-Pyridyl)-N-oxide	3-Phenylpropyl	3,4,5-Trimethoxyphenyl
23	2	0	3-[N-(7-azaindolyl)-propyl	3-Phenylpropyl	3,4,5-Trimethoxyphenyl
24	2	0	3-(3-Pyridyl)-propyl	3-(4-Methoxyphenyl)propyl	3,4,5-Trimethoxyphenyl

Table 1 (continued)

No.	n	m	B	D	L
25	2	0	3-(N-purinyl)-propyl	3-Phenylpropyl	3,4,5-Trimeth-oxyphenyl
26	2	0	3-(4-hydroxymethyl-phenyl)propyl	3-Phenylpropyl	3,4,5-Trimeth-oxyphenyl
27	2	0	3-(3-Pyridylpropyl)	3-Phenylpropyl	3-Benzyloxy-phenyl
28	2	0	3-(3-Pyridylpropyl)	3-Phenylpropyl	3-Allyloxyphenyl
29	2	0	3-(3-Pyridylpropyl)	3-Phenylpropyl	3-Isopropoxy-phenyl
30	2	0	3-(2-Thienyl)propyl	3-Phenylpropyl	3,4,5-Trimeth-oxyphenyl
31	2	0	3-(4-Carboxyphenyl)-propyl	3-Phenylpropyl	3,4,5-Trimeth-oxyphenyl
32	2	0	4-Phenylbutyl	3-Phenylpropyl	3,4,5-Trimeth-oxyphenyl
33	2	0	2-Hydroxymethyl-phenyl	3-Phenylpropyl	3,4,5-Trimeth-oxyphenyl
34	2	0	2-Allyloxyphenyl	3-Phenylpropyl	3,4,5-Trimeth-oxyphenyl
35	2	0	3-(3-Hydroxymethyl-phenyl)propyl	3-Phenylpropyl	3,4,5-Trimeth-oxyphenyl
36	2	0	3-(3-Carboxyphenyl)-propyl	3-Phenylpropyl	3,4,5-Trimeth-oxyphenyl
37	2	0	3-Hydroxymethyl-phenyl	3-Phenylpropyl	3,4,5-Trimeth-oxyphenyl
38	2	0	2-Hydroxyphenyl	3-Phenylpropyl	3,4,5-Trimeth-oxyphenyl
39	2	0	3-Pyridyl	3-Phenylpropyl	3,4,5-Trimeth-oxyphenyl

Table 1 (continued)

No.	n	m	B	D	L
40	2	0	3-(2-Thienyl)propyl	4-Phenylbutyl	3,4,5-Trimethoxyphenyl
41	2	0	5-Phenylpentyl	3-Phenylpropyl	3,4,5-Trimethoxyphenyl

TABLE 2: Assay Results

No.	K_i (nM)	K_d (nM)	PMA (μM)	OKT3 (μM)	LB (μM)	JVM (μM)	CTLL (μM)
2	95	ND	3.5	1.5	8.0	7.0	4.0
3	1.0	20	>8	2.25	10	8.5	8.0
4	220	ND	>10	>10	>10	>10	>10
5	4000	ND	>10	>10	>10	>10	8.0
6	80	40	8.0	8.0	10	9.0	5.0
7	3.0	29	2.0	2.0	5.0	5.0	2.0
8	27	30	4.0	5.0	10	10	4.0
9	1.0	4.0	1.8	1.2	6.0	6.0	2.5
10	32	ND	5.5	4.0	5.5	8.0	6.0
11	24	ND	2.7	2.0	3.5	6.5	10
12	83	ND	7.0	5.0	10	10	8.1
13	4.0	27	8.0	10	>10	>10	9.0
14	270	ND	>10	>10	>10	>10	>10
15	ND	ND	3.0	3.0	6.0	8.0	5.0
16	57	ND	5.0	7.0	9.0	>10	6.0
17	3.0	68	4.5	2.5	5.5	7.0	>10

Table 2 (continued)

No.	K_i (nM)	K_d (nM)	PMA (μM)	OKT3 (μM)	LB (μM)	JVM (μM)	CTLL (μM)
18	56	ND	5.0	7.0	8.0	9.0	7.0
19	50	ND	6.0	8.0	10	10	5.0
20	1.0	4.0	1.7	2.0	2.0	2.5	2.0
21	36	ND	7.0	3.0	6.0	7.0	4.0
22	ND	ND	1.5	1.6	2.2	2.5	2.3
23	ND	ND	5.0	7.0	7.0	6.0	3.5
24	ND	ND	ND	ND	ND	ND	ND
25	9.0	ND	ND	2.0	2.5	2.0	3.0
26	4.0	19	3.0	1.0	3.0	2.5	4.0
27	15	ND	6.0	4.0	10	6.0	5.0
28	11	ND	5.0	6.0	8.0	9.0	3.0
29	2.0	3.0	10	8.0	>10	>10	4.0
30	4.0	90	5.0	2.5	8.0	6.0	7.0
31	2.0	8.0	5.0	7.0	6.0	6.0	7.0
32	20	ND	9.0	8.0	>10	>10	7.5
33	89	ND	5.0	5.0	2.0	4.5	3.0
34	>150	ND	3.0	9.0	4.0	3.0	10
35	1.0	40	2.7	6.5	2.5	3.0	2.5
36	4.0	20	>10	>10	>10	9.0	>10
37	7.0	140	2.0	5.0	1.2	2.2	1.2
38	ND	ND	>10	5.0	>10	>10	>10

Table 2 (continued)

No.	K_i (nM)	K_d (nM)	PMA (μM)	OKT3 (μM)	LB (μM)	JVM (μM)	CTLL (μM)
39	10	ND	6.0	5.5	ND	5.0	2.5
40	1100	ND	>10	>10	>10	>10	10
41	45	ND	7.0	>10	>10	10	>10

All of the compounds in Table 2 showed toxicity at higher concentrations than their immunosuppresive activity and were typically concentrations >10 μM.
K_i - inhibition of FKBP rotamase activity
K_D - binding to FKBP
PMA and OKT3 - mitogens used to stimulate proliferation of human peripheral blood lymphocytes (PBC). Compounds are evaluated on their ability to inhibit proliferation.
LB and JVM - human viral-transformed B lymphoblastoid cell lines stimulated to proliferate in a mixed lymphocyte reaction (MLR). The compounds are evaluated on their ability to inhibit this proliferation.
CTLL - inhibition of proliferation of cytotoxic T cells stimulated by IL-2
ND - not determined.

The immunosuppressive compounds of this invention have an affinity for the FK-506 binding protein which is located in the cytosol of lymphocytes, particularly T lymphocytes. When the immunosuppressive compounds are bound to the FKBP, they act to inhibit the prolyl-peptidyl cis-trans isomerase activity of the binding protein and inhibit lymphocyte activation mediated by FKBP. One particular FK-506 binding protein has been identified by Harding, M.W. et al., Nature 341:758-760 (1989) and can be used as the standard by which to evaluate binding affinity of the compounds for FKBP. Compounds of this invention, however, may have an

affinity for other FK-506 binding proteins. Inhibition of the prolyl peptidyl cis-trans isomerase may further be indicative of binding to an FK-506 binding protein.

Human FK-506 binding protein can be obtained as described by Harding, M.W. et al., Nature 341:758-760 (1989). Values for the apparent K_d can be determined from a competitive LH-20 binding assay performed as described by Harding et al., using 32-[1-^{14}C]-benzoyl FK-506 as a reporting ligand; or using [^{3}H]dihydro-FK-506, as described by Siekierka, J.J. et al., Nature 341:755-757 (1989). The binding affinities for several compounds of this invention for the FKBP are reported in Table 2. The data was obtained using the latter method, where the ability of an unlabeled compound to compete with the binding of [^{3}H]dihydro-FK-506 to FK-506 binding protein was measured.

The inhibition of the PPIase (rotamase) enzyme activity of the FKBP (apparent "K_i" values) can also be measured according to the methods described by either Harding, M.W. et al., Nature 341:758-760 (1989) or Siekierka, J.J. et al., Nature 341:755-757 (1989). The cis-trans isomerization of the proline-alanine peptide bond in a model substrate, N-succinyl-Ala-Ala-Pro-Phe-p-nitroanilide, is monitored spectrophotometrically in a coupled assay with chymotrypsin, which releases 4-nitro-anilide from the trans form of the substrate. Fischer, G. et al., Nature 337:476-478 (1989). The inhibitory effect of the addition of different concentrations of inhibitor on the extent of the reaction is determined, and analysis of the change in first order rate constant as a function of inhibitor concentration yields an estimate of the apparent K_i value. The extent of enzyme

inhibition (K_i) of some preferred compounds is shown in Table 2.

The compounds of the present invention can be further characterized in cellular biological experiments in vitro where their resemblance in function and use to cyclosporin A and to FK-506 is apparent. (See Table 3).

TABLE 3

Assays and IC_{50} Value for Drugs	Cyclosporin A	Rapamycin	FK-506
1) Human PBL + OKT3	<1μg/ml	<1μg/ml	<1μg/ml
2) T-Cell Hybridoma + TCR/CD2	<1μg/ml	<1μg/ml	<1μg/ml
3) Apoptosis	Blocks at 1μg/ml	Inactive at 1μg/ml	Blocks at 1μg/ml
4) CTLL Proliferation + IL-2	>>1μg/ml	≈0.01μg/ml	>>1μg/ml

1) Assay similar to Yoshimura, N. et al., Transplantation 47:356-359 (1989). Assay uses fresh human peripheral blood lymphocytes isolated by Ficoll-Hypaque density centrifugation, stimulated by the OKT3 antibody (anti-CD3) which stimulates via interaction with CD3. Stimulation is measured by incorporation of radioactive thymidine [(^{3}H)TdR] into proliferating cells, with an uninhibited control signal of 48,000-75,000 cpm. IC_{50} values are estimated from inhibition of proliferation observed at various drug concentrations.

2) Assay similar to above, but using T-cell clone stimulated with antibody to the T-cell receptor (TCR) and

antibody to CD2. Stimulation is measured by incorporation of radioactive thymidine [(^{3}H)TdR] into proliferating cells, with an uninhibited control signal of 23,000 cpm. IC_{50} values are estimated from inhibitions of proliferation observed at various drug concentrations.

3) Assay according to Shi, Y. et al., Nature 339:625-626 (1989). The assay uses a T-cell hybridoma similar to that described. The assay measures activation-induced (anti-CD3) cell death (evaluated by counting viable cells after staining as described) in a T-cell hybridoma that mimics the effect known to occur in immature thymocytes. The ability of cyclosporin A and FK-506 to inhibit this cell death is herein used as a sensitive indication of compounds with cyclosporin-like and/or FK-506-like mechanism of action. Note that the chemically related, but mechanistically distinct, immunosuppressant rapamycin is inactive in this assay.

4) Assay according to DuMont, F. et al., J. Immunol. 144:251-258 (1990). The assay measures the stimulation of CTLL cells in response to IL-2. Proliferation is measured by incorporation of (^{3}H)TdR. Immunosuppressants which work by a similar mechanism to cyclosporin A and FK-506 will not inhibit in this IL-2 driven process, since they function by the inhibition of production of endogenous IL-2. In this assay, exogenous IL-2 is provided to overcome this block. Note that the chemically related, but mechanistically distinct immunosuppressant, rapamycin, is active in this assay.

These assays and the ones set forth in the Example Section can be used to profile the cellular activity of the compounds of the present invention. Thus, it is clear from these results that the compounds of this

invention resemble both cyclosporin A and FK-506 in its cellular activity, including immunosuppression, in contrast to the mechanistically dissimilar immunosuppressant agent rapamycin. Furthermore, the observed cellular activity is consistent quantitatively with the activity observed for FKBP binding and inhibition of PPIase (rotamase) activity shown in Table 2. Thus, the compounds can be used as immunosuppressants for prophylaxis of organ rejection or treatment of chronic graft rejection and for the treatment of autoimmune diseases.

The immunosuppressive compounds of this invention can be periodically administered to a patient undergoing bone marrow or organ transplantation or for another reason in which it is desirable to substantially reduce or suppress a patient's immune response, such as in various autoimmune diseases. The compounds of this invention can also be administered to mammals other than humans for treatment of various mammalian autoimmune diseases.

The novel compounds of the present invention possess an excellent degree of activity in suppression of antigen-stimulated growth and clonal expansion of T-cells, especially those T-cells characterized as "helper" T-cells. This activity is useful in the primary prevention of organ transplant rejection, in the rescue of transplanted organs during a rejection episode, and in the treatment of any of several autoimmune diseases known to be associated with inappropriate autoimmune responses. These autoimmune diseases include: uveitis, Behcet's disease, Graves ophthalmopathy, psoriasis, acute dermato-

myositis, atopic skin disease, scleroderma, eczema, pure red cell aplasia, aplastic anemia, primary cirrhosis, autoimmune hepatitis, ulcerative colitis, Crohn's disease, amyotrophic lateral sclerosis, myasthenia gravis, multiple sclerosis, nephrotic syndrome, membrano-proliferative glomerulonephritis, rheumatoid arthritis and insulin-dependent diabetes mellitus. In all of the above-listed autoimmune diseases, treatment is effective to reduce the symptoms and slow progression of the disease. In the case of insulin-dependent diabetes mellitus, treatment as described below is most effective when instituted before the complete cessation of natural insulin production and transition to complete dependence on external insulin.

For these purposes the compounds of the present invention may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir in dosage formulations containing conventional non-toxic pharmaceutically-acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes subcutaneous, intravenous, intramuscular, intrasternal and intracranial injection or infusion techniques.

The pharmaceutical compositions may be in the form of a sterile injectable preparation, for example as a sterile injectible aqueous or oleagenous suspension. This suspension may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butanediol.

Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or di-glycerides. Fatty acids such as oleic acid and its glyceride derivatives find use in the preparation of injectables, as do natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant such as Ph. Helv or similar alcohol.

The compounds may be administered orally, in the form of capsules or tablets, for example, or as an aqueous suspension or solution. In the case of tablets for oral use, carriers which are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening and/or flavoring and/or coloring agents may be added.

The compounds of this invention may also be administered in the form of suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at room temperature but liquid at the rectal temperature and therefore will melt in the rectum to release the drug. Such materials include cocoa butter, beeswax and polyethylene glycols.

The compounds of this invention may also be administered topically, especially when the conditions addressed for treatment involve areas or organs readily accessible by topical application, including autoimmune diseases of the eye, the skin, or the lower intestinal tract. Suitable topical formulations are readily prepared for each of these areas.

For ophthalmic use, the compounds can be formulated as micronized suspensions in isotonic, pH adjusted sterile saline, or, preferably, as solutions in isotonic, pH adjusted sterile saline, either with or without a preservative such as benzylalkonium chloride. Alternatively for the ophthalmic uses, the compounds may be formulated in an ointment such as petrolatum.

For application topically to the skin, the compounds can be formulated in a suitable ointment containing the compound suspended or dissolved in, for example, a mixture with one or more of the following: mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax and water. Alternatively, the compounds can be formulated in a suitable lotion or cream containing the active compound suspended or dissolved in, for example, a mixture of one or more of the following: mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

Topical application for the lower intestinal tract can be effected in a rectal suppository formulation (see above) or in a suitable enema formulation.

Dosage levels on the order of 0.01 to 100 mg/kg per day of the active ingredient compound are useful in the treatment of the above conditions. The amount of active

ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration.

It is understood, however, that a specific dose level for any particular patient will depend upon a variety of factors, including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, rate of excretion, drug combination and the severity of the particular disease being treated.

The compound can also be administered in combination with a steroid, such as methyl prednisalone acetate, for additional immunosuppressive effect. The steroid is administered orally, intravenously, rectally, topically or by inhalation. Dosages (based upon methyl prednisalone acetate) of 0.1-5 mg/kg/day may be employed. An initial loading dose of 100-500 mg may be employed. Steroid doses may be decreased with time from the higher toward the lower doses as the clinical situation indicates.

The compounds can be administered with other immunosuppressant drugs, such as rapamycin, azathioprine, 15-deoxyspergualin, cyclosporin, FK-506 or combinations of these, to increase the immunosuppressive effect. Administration of cyclosporin and FK-506 together should be avoided due to contraindications reported resulting from coadministration of these immunosuppressants. The dosage level of other immunosuppressant drugs will depend upon the factors previously stated and the immunosuppressive effectiveness of the drug combination.

OKT3, which is a murine monoclonal antibody to CD3 surface antigen of human T lymphocytes, can also be coadministered intravenously with compounds of the

present inventions for rescue and reversal of acute allograft rejections, particularly in renal transplantations.

The invention will be further illustrated by way of the following examples, which are not intended to be limiting in any way.

EXAMPLES

General

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded at 500 MHz on a Bruker AMX 500. Chemical shifts for proton resonances are reported in parts per million (δ) relative to Me_4Si (δ 0.0). Analytical high performance liquid chromatography (HPLC) was performed on either a Waters 600E or a Hewlett Packard 1050 liquid chromatograph.

The compounds described below are illustrated in Figure 1.

EXAMPLE 1

Synthesis of (S)-1,7-Diphenyl-4-heptanyl N-(3,4,5-trimethoxyphenylglyoxyl)pipecolate (3)

4-Phenyl-1-butyraldehyde (26)

To a solution of 3.2 mL (20.8 mmol) of 4-phenyl-1-butanol (Aldrich Chemical Co.) in 20 mL of CH_2Cl_2 at 0°C was added 3.2 g of powdered 3 A molecular sieves and then 5.37 g (24.9 mmol) of pyridinium chlorochromate (PCC). The resulting suspension was stirred at 0°C for 1 h at which time an additional 2.16 g (10.0 mmol) of PCC was added and the reaction mixture was

warmed to room temperature. After stirring at ambient temperature for 0.5 h, the reaction mixture was diluted with ether and filtered through celite to give 2.5 g of the crude product. Flash chromatography (elution with 5% ethyl acetate in hexane) yielded 700 mg of the aldehyde (26). ^{1}H NMR consistent with the product.

3-Phenyl-1-propylmagnesium bromide (27)

To a suspension of 736 mg (30.3 mmol) of magnesium turnings in 50 mL of THF at room temperature was added 50 μL of 1,2-dibromoethane followed by the dropwise addition of 5.5 g (25.1 mmol) of 1-bromo-3-phenylpropane (Aldrich Chemical Co.). After stirring at room temperature for 0.5 h, the supernatent was transfered via canula to a 100 mL storage vessel and subsequently used as a 0.5 M THF solution of the Grignard reagent (27).

1,7-Diphenyl-4-heptanol (28)

To a solution of 700 mg (4.7 mmol) of 4-phenyl-1-butanal (26) in 5.0 mL of THF at 0°C was added 10.0 mL (5.0 mmol) of 3-phenyl-1-propylmagnesium bromide (27) and the resulting mixture was stirred at 0°C for 0.5 h. The mixture was then quenched by the dropwise addition of saturated NH_4Cl and diluted with ether. The phases were separated and the organic layer was washed with water and brine and then dried over $MgSO_4$. Concentration gave 1.12 g of the alcohol (28) as an oil. The ^{1}H NMR spectrum of this compound was consistent with the structure.

(S)-Boc-Pipecolyl-1,7-dipenyl-4-heptanyl ester (29)

To a solution of 164.2 mg (0.72 mmol) (S)-Boc-pipecolic acid in 5.0 mL of CH_2Cl_2 at room temperature was added 174.7 mg (0.65 mmol) of alcohol (28), 140.8 mg (0.72

mmol) of 1-(3-dimethylaminopropyl)3-ethylcarbodiimide hydrochloride (EDC) and a catalytic amount of N,N-dimethylaminopyridine (DMAP). The reaction mixture was stirred at ambient temperature for 0.5 h and then applied directly to a silica gel column. Elution with 10% ethyl acetate in hexane afforded 76.2 mg of the ester (29) as an oil. ^{1}H NMR consistent with the product.

(S)-1,7-Diphenyl-4-heptanylpipecolate (30)

To a solution of 47 mg (0.10 mmol) of (29) in 1.0 mL of CH_2Cl_2 at ambient temperature was added 1.0 mL of trifluoroacetic acid. After stirring at room temperature for 0.5 h, the resulting solution was neutralized by the dropwise addition of saturated K_2CO_3. The layers were separated and the organic phase was washed with water, dried over $MgSO_4$ and concentrated to yield 23 mg of the amine (30) as an oil. ^{1}H NMR consistent with structure.

3,4,5-Trimethoxybenzoylformic acid (31)

To a solution of 9.2 g (43.4 mmol) of 3,4,5-trimethoxyacetophenone (Aldrich Chemical Co.) in 35 mL of pyridine was added 6.3 g (56.7 mmol) of selenium dioxide and the resulting solution was heated at reflux overnight. The reaction mixture was cooled to room temperature, filtered through celite and concentrated to yield a dark brown oil which was dissolved into ethyl acetate and washed with 1.0 N HCL and then with saturated $NaHCO_3$. The basic aqueous layer was diluted with ether and acidified with concentrated HCl. The layers were separated and the organic phase was washed with brine and then dried over Na_2SO_4 to give 8.4 g of a dark yellow

solid. Recrystalization of this material from ethyl acetate-hexane then gave 6.8 g of the acid (31) as a pale yellow solid. ^{1}H NMR was consistent with the structure.

(S)-1,7-Diphenyl-4-heptanyl N-(3,4,5-trimethoxyphenylglyoxyl)pipecolate (3)

To a solution of 23 mg (0.06 mmol) of the amine (30) in 1.0 mL of CH_2Cl_2 at room temperature was added 21.8 mg (0.09 mmol) of the acid (31) and then 17.9 mg (.09 mmol) of EDC and the resulting solution was stirred at room temperature for 0.5 h and applied directly to a silica gel column. Elution with 15% ethyl acetate in hexane gave 8.4 mg of the amide (3) as a mixture of rotamers. ^{1}H NMR (500 MHz, $CDCl_3$)δ 7.35-7.06(m), 5.32(br s),5.00(br s), 4.88(br s), 4.58(d), 4.31(br s), 3.95(s), 3.90(s), 3.89(s), 3.85(s), 3.44(d), 3.21(t), 3.04(t), 2.54(br s), 2.51 (br s), 2.42(br s), 2.30(d), 2.15(d), 1.83-1.21(m).

EXAMPLE 2

Synthesis of (R and S)-1-(3-Phenoxy)phenyl-4-phenyl-1-butyl (S)-N-(3,4,5-trimethoxyphenylglyoxyl)-pipecolate (4)

3-Phenoxybenzaldehyde (32)

To a solution of 1.8 mL (10.3 mmol) of 3-phenoxybenzyl alcohol (Aldrich Chemical Co.) in 20 mL of CH_2Cl_2 at room temperature was added 1.5 g of powdered 4 A molecular sieves and 2.5 g of activated MnO_2. The resulting suspension was stirred at room temperature for 0.5 h at which time an additional 2.5 g of MnO_2 was added. After stirring at room temperature for 0.5 h the

reaction mixture was filtered through celite to give 1.84 g of the aldehyde (32) as an oil. ^{1}H NMR consistent with structure.

(R and S)-1-(3-Phenoxy)phenyl-4-phenyl-1-butanol (33)

The alcohol (33) was prepared from 190 mg (0.96 mmol) of aldehyde (32) and 2.0 mL (1.0 mmol) of (27) in 2.0 mL of THF as described above for the synthesis of (28) in Example 1. Flash chromatography (elution with 10% ethyl acetate in hexane) afforded 108 mg of the racemic alcohol (33). ^{1}H NMR consistent with structure.

(S)-N-3,4,5-(Trimethoxyphenyl)glyoxylpipecolic acid (34)

To a slurry of 953.3 mg (3.4 mmol) of the tartrate salt of (S)-pipecolic acid (Egbertson, M. and S.J. Danishefsky, J. Org. Chem. 54:11 (1989)) in 7.0 mL of CH_2Cl_2 at 0°C was added 3.9 mL (22.4 mmol) of diisopropylethylamine and 2.4 mL (18.9 mmol) of chlorotrimethylsilane and the resulting solution was allowed to stir at 0°C for 0.5 h. In a separate reaction flask 450 μL (5.2 mmol) of oxalyl chloride and three drops of DMF was added to a solution of 820 mg (3.4 mmol) of acid (31) in 7.0 mL of CH_2Cl_2. After the evolution of gas ceased, the entire contents of the second flask were added to the first reaction vessel and the resulting mixture was allowed to stir at room temperature for 1 h. The reaction mixture was concentrated, dissolved into ether and washed with 0.5 N HCl and then saturated $NaHCO_3$. The basic aqueous phase was acidified with concentrated HCl and extracted with ether. The ethereal

extracts were washed with water, brine, dried over $MgSO_4$ and concentrated to give 490 mg of the acid (34). ^1H NMR consistent with structure.

(R and S)-1-(3-Phenoxy)phenyl-4-phenyl-1-butyl (S)-N-(3,4,5-trimethoxyphenylglyoxyl)pipecolate (4)

To a solution of 29.4 mg (0.08 mmol) of acid (34) in 2.0 mL of CH_2Cl_2 at room temperature was added 11 μL (0.13 mmol) of oxalyl chloride and three drops of DMF and the reaction mixture was allowed to stir at room temperature for 0.5 h and was then concentrated and suspended in 1.0 mL of benzene. To this suspension was added 32.0 mg (0.1 mmol) of alcohol (33) and 13.4 mg (0.1 mmol) of silver cyanide. The resulting mixture was heated at reflux overnight, cooled to room temperature and concentrated. Flash chromatography (elution with 10% ethyl acetate in hexane) gave 8.8 mg of the ester (4) as a mixture of diastereomers. ^1H NMR (500 MHz, $CDCl_3$)δ 7.34-7.19(m), 7.18-7.03(m), 7.02-6.84(m), 6.83-6.72(m), 5.73(q), 5.69-5.55(m), 5.38(t), 4.55(br d), 4.35(dd), 3.94(s), 3.92(s), 3.89(s), 3.83(s), 3.73(s), 3.63(s), 3.48-3.35(m), 3.20(t), 3.10(t), 2.60(q), 2.40(dd), 1.95-1.91(m), 1.90-1.45(m).

EXAMPLE 3

Synthesis of (R and S)-6-Phenyl-1-(3-pyridyl)-3hexyl (S)-N-(3,4,5-trimethoxyphenylglyoxyl)pipecolate (7)

3-(3-Pyridyl)-1-propylaldehyde (35)

To a solution of 2.3 g (5.46 mmol) of the Dess-Martin periodinane (Dess, D.B. and J.C. Martin, J. Org. Chem. 48:4155 (1983)) in 10 mL of CH_2Cl_2 at 0°C was

added 470 μL (3.65 mmol) of 3-(3-pyridyl)-1-propanol and the resulting mixture was allowed to warm from 0°C to ambient temperature over a 1.5 h period. To this solution was added 6.0 g (38.22 mmol) of $Na_2S_2O_3$ in saturated $NaHCO_3$ and the reaction mixture was allowed to stir at room temperature for 15 min. The reaction was extracted with CH_2Cl_2, dried over $MgSO_4$ and concentrated. Flash chromatography (elution with 3:1 hexane:acetone) yielded the product aldehyde (35) as an oil. ^1H NMR consistent with structure.

(R and S)-6-Phenyl-1-(3-pyridyl)-3-hexanol (36)

The alcohol (36) was prepared from 125 mg (0.92 mmol) of aldehyde (35) and 2.0 mL (1.0 mmol) of (27) in 2.0 mL of THF as described above for the synthesis of (28) in Example 1 to give 221 mg of the crude alcohol (36). ^1H NMR consistent with structure.

(S)-Boc-Pipecolyl-(R and S)-6-Phenyl-1-(3-pyridyl)-3-hexyl ester (37)

The ester (37) was prepared from 125 mg (0.49 mmol) of alcohol (36), 93 mg (0.41 mmol) of (S)-Boc-pipecolic acid, 94 mg (0.49 mmol) of EDC and a catalytic amont of DMAP in 1.0 mL of CH_2Cl_2 and 1.0 mL of DMF as described above for the synthesis of (29) in Example 1. Flash chromatography (elution with 2:1 hexane:ethyl acetate) gave 105 mg of the diastereomeric ester (37) as an oil. ^1H NMR consistent with structure.

(R and S)-6-Phenyl-1-(3-pyridyl)-3-hexyl (S)-pipecolate (38)

The amine (38) was synthesized by treating 95 mg (0.20 mmol) of the ester (37) with 1.0 mL of

trifluoroacetic acid in 3.0 mL of CH_2Cl_2 as described above for the preparation of (30) in Example 1 giving 58 mg of the diastereomeric amine (38) as an oil. ^1H NMR consistent with structure.

(R and S)-6-Phenyl-1-(3-pyridyl)-3-hexyl (S)-N-(3,4,5-trimethoxyphenylglyoxyl)pipecolate (7)

The ester (7) was prepared from 54 mg (0.15 mmol) of the amine (38), 50 mg (0.22 mmol) of the acid (31) and 42 mg (0.22 mmol) of EDC in 3.0 mL of CH_2Cl_2 as described above in the synthesis of ester (3) in Example 1. Flash chromatography (elution with 1:1 ethyl acetate:hexane) gave 73 mg of the diasteromeric ester (7) as a mixture of rotamers. ^1H NMR (500 MHz $CDCl_3$)δ 8.48-8.42(m), 7.50-7.41(m), 7.32(d), 7.27-7.03(m), 5.38(d), 5.31(d), 5.06-5.01(m), 4.97-4.93(m), 4.60(br d), 3.92(s), 3.88(s), 3.86(s), 3.84(s), 3.82(s), 3.79(s), 3.46(br d), 3.27(br t), 2.73-2.68(m), 2.38-2.29(m), 1.98-1.76(m), 1.75-1.60(m), 1.56-1.51(m), 1.38-1.20(m).

EXAMPLE 4

Synthesis of (R and S)-(E)-1-[trans-(4-Hydroxycyclohexyl)]-2-methyl-6-phenyl-3-hex-1-enyl (S)-N-(3,4,5-trimethoxyphenylglyoxyl)pipecolate (8)

cis- and trans-4-(tert-Butyldimethylsilyloxy) cyclohexan-1-ol (39) and (40)

To a solution of 3.43 g (21.7 mmol) of cis- and trans-methyl 4-hydroxycyclohexane carboxylate (Noyce, D.S. and D.B. Denney, J. Am. Chem. Soc. 74:5912 (1952)) in 45 mL of methylene chloride at 0°C was added 3.0 mL (26.0 mmol) of 2,6-lutidine followed by 5.5 mL (23.8)

mmol of tert-butyldimethylsilyl trifluoromethanesulfonate. The ice bath was removed and the reaction mixture was allowed to stir at 25°C for 2 h at which time the solution was poured into saturated sodium bicarbonate. The layers were partitioned and the organic layer was washed with saturated copper sulfate and water and then dried over $MgSO_4$ to give 5.9 g of the crude methyl esters. A solution of 5.72 g (21.0 mmol) of this mixture in 45 mL of anhydrous THF was treated with 400 mg (10.5 mmol) of lithium aluminum hydride. The reaction mixture was stirred at 25°C for 0.5 h and was then quenched by the slow addition of a saturated solution of Rochelle's salt. The mixture was diluted with ether, the layers were partitioned and the aqueous layer was washed twice with ethyl acetate. The combined organic extracts were dried over $MgSO_4$ and concentrated to give 4.9 g of the diastereomeric alcohols. Flash chromatography (elution with 1:5 ethyl acetate-hexane) gave 650 mg of (39), 1.10 g of (40) and 2.40 g of a mixture of the two. Data for (39): ^1H NMR (300 MHz, $CDCl_3$) δ 3.99-3.92 (m), 3.46 (d), 1.72-1.58 (m), 1.57-1.36 (m), 0.86 (s), 0.08 (s). Data for (40): ^1H NMR (300 MHz, $CDCl_3$)δ 3.47 (dddd), 3.38 (d), 1.86-1.67 (m), 1.47-1.16 (m), 1.05-0.77 (m), 0.72 (s), 0.02 (s).

(E)-Ethyl 3-[trans-(4-tert-Butyldimethyl-silyloxycyclohexyl)]-2-methylprop-2-enoate (41)

To a -78°C solution of oxalyl chloride (785 μL, 9.0 mmol) in 10 mL of methylene chloride was added dimethylsulfoxide (1.3 mL, 18.0 mmol). The resulting solution was stirred for 5 min and then 1.1 g (4.5 mmol)

of the alcohol (40) was added in 10 mL of methylene chloride. The reaction mixture was stirred at -78°C for 45 min at which time 3.8 mL (27.0 mmol) of triethylamine was added and the solution was allowed to warm to ambient temperature. The reaction was quenched with 1.0 N HCl and the aqueous layer was extracted with three portions of methylene chloride. The combined organic extracts were dried over $MgSO_4$ and evaporated to dryness to give 1.0 g of the intermediate aldehyde. A solution of this aldehyde (450 mg, 1.86 mmol) was treated directly with 710 mg (1.95 mmol) of (carbethoxyethylidene)triphenylphosphorane in 5.0 mL of methylene chloride. The resulting reaction mixture was stirred at ambient temperature overnight and was then poured into water. The layers were partitioned and the aqueous layer was extracted twice with methylene chloride. The combined organic layers were dried over $MgSO_4$ and concentrated to yield the enoate (41) containing a minor amount of the Z isomer. ^1H NMR consistent with structure.

(E)-3-[trans-(4-tert-Butyldimethylsilyloxy-cyclo-hexyl)]-2-methylprop-2-en-1-ol (42)

To a solution of 860 mg (2.6 mmol) of enoate (41) in 5.0 mL of anhydrous tetrahydrofuran at 25°C was added 50 mg (1.3 mmol) of lithium aluminum hydride and the resulting mixture was allowed to stir for 30 min. The reaction was quenched by the slow addition of saturated Rochelle's salt and diluted with ethyl acetate. The layers were separated and the aqueous layer was extracted with two portions of ethyl acetate. The combined organic extracts were washed with water and brine and then dried over $MgSO_4$. Evaporation and flash chromatography

(elution with 15% ethyl acetate in hexane) gave 370 mg of the allylic alcohol (42). ^{1}H NMR consistent with structure.

(E)-3-[trans-(4-tert-Butyldimethylsilyloxy-cyclo-hexyl)]-2-methylprop-2-en-1-ol (43)

To a -78°C solution of oxalyl chloride (105 μL, 1.2 mmol) in 1.0 mL of methylene chloride was added dimethylsulfoxide (170 μL, 2.4 mmol). The resulting solution was stirred for 5 min and then 170 mg (0.6 mmol) of the alcohol (42) was added in 1.0 mL of methylene chloride. The reaction mixture was stirred at -78°C for 45 min at which time 500 μL (3.6 mmol) of triethylamine was added and the solution was allowed to warm to ambient temperature. The reaction was quenched with 1.0 N HCl and the aqueous layer was extracted with three portions of methylene chloride. The combined organic extracts were dried over $MgSO_4$ and evaporated to dryness to give the crude aldehyde (43) which was used directly in the next reaction. ^{1}H NMR consistent with structure.

(R and S)-(E)-1-[trans-(4-tert-Butyldimethyl-silyloxycyclohexyl)]-2-methyl-6-phenyl-3-hex-1-en-3-ol (44)

The alcohol (44) was prepared from the crude aldehyde (43) and 1.5 mL (.075 mmol) of (27) in 2.0 mL of THF as described above for the synthesis of (28) in Example 1 to give 220 mg of the crude diastereomeric alcohol (44). Flash chromatography (elution with 20% ethyl acetate in hexane) afforded 146 mg of the alcohol (44) as an oil. ^{1}H NMR consistent with structure.

(R and S)-(E)-1-[trans-(4-tert-Butyldimethylsilyloxycyclohexyl)]-2-methyl-6-phenyl-3-hex-1-enyl (S)-N-(3,4,5-trimethoxyphenylglyoxyl)pipecolate (45)

To a solution of 75.7 mg (0.22 mmol) of acid (34) in 2.5 mL of CH_2Cl_2 at room temperature was added 30 μL (0.34 mmol) of oxalyl chloride and three drops of DMF and the reaction mixture was allowed to stir at room temperature for 0.5 h and was then concentrated and suspended in 1.0 mL of benzene. To this suspension was added 43.4 mg (0.11 mmol) of alcohol (44) and 28.8 mg (0.22 mmol) of silver cyanide. The resulting mixture was heated at reflux overnight, cooled to room temperature and concentrated. Flash chromatography (elution with 4% acetone in hexane) gave 17.5 mg of the ester (45) as a mixture of diastereomers. ^1H NMR consistent with structure.

(R and S)-(E)-1-[trans-(4-Hydroxycyclohexyl)]-2-methyl-6-phenyl-3-hex-1-enyl (S)-N-(3,4,5-trimethoxyphenylglyoxyl)pipecolate (8)

To a solution of 17.5 mg (0.02 mmol) of the ester (45) in 1.0 mL of CH_3CN at room temperature was added 10 drops of a 95:5 solution of CH_3CN:5% HF and the resulting mixture was stirred at room temperature for 0.5 h. The reaction mixture was neutralized with saturated K_2CO_3 and extracted into ether. The ether layers were washed with water, dried over $MgSO_4$ and concentrated to yield 7.2 mg of crude material. Flash chromatography (elution with 15% acetone in hexane) gave 4.9 mg of the diastereomeric alcohol (8) as a mixture of rotamers. ^1H NMR (500 MHz, $CDCl_3$)δ 7.38-7.02(m), 5.35-5.01(m), 4.62-4.53(m),

4.28(t), 3.95(s), 3.89(s), 3.87(s), 3.86(s), 3.85(s), 3.81(s), 3.55(m), 3.45(m), 3.20(m), 3.10-2.90(m), 2.60-2.45(m), 2.32(t), 2.10(t), 1.95(d), 1.85-1.40(m), 1.39-1.02(m).

EXAMPLE 5

Synthesis of (R and S)-5-(3-indolyl)-phenyl-2-pentyl (S)-N-(3,4,5-trimethoxyphenylglyoxyl)pipecolate (11)

N-Methyl-N-Methoxy-4-(3-indolyl)butyramide (46)

To a slurry of 1.75 g (8.61 mmol) of 3-indolebutyric acid (Aldrich Chemical Co.) in acetonitrile at room temperature was added 7.0 mL (40.2 mmol) of N,N-diisopropylethylamine, 1.0 g (10.3 mmol) of N,N-dimethylhydroxylamine hydrochloride and 4.19 g (9.5 mmol) of benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent) and the resulting mixture was allowed to stir at room temperature overnight and was then concentrated to dryness. The residue was dissolved into ethyl acetate and washed with water, 0.5 N HCl, saturated $NaHCO_3$ and brine and then dried over $MgSO_4$ and concentrated. Flash chromatography (elution with a gradient of 2-10% ether in methylene chloride) provided 2.0 g of the amide (46). ^1H NMR consistent with structure.

Benzyl-3-(3-indolyl)propyl ketone (47)

To a solution of 147 mg (0.60 mmol) of amide (46) in 4.0 mL of THF at -78°C was added 1.31 mL (1.31 mmol) of benzylmagnesium chloride (1.0 M in Et_2O) and the reaction mixture was allowed to warm to room temperature and stir for 3 h. The reaction was quenched with 5% $KHSO_4$ and

extracted into ether. The combined ethereal layers were washed with brine and dried over $MgSO_4$. Flash chromatography (elution with 25% ether in hexane) gave 108 mg of the ketone (47). ^1H NMR consistent with structure.

(R and S)-5-(3-indolyl)-1-phenyl-2-pentanol (48)

To a slurry of 105 mg (0.38 mmol) of ketone (47) in 3.0 mL of MeOH at 0°C was added 30 mg (0.79 mmol) of solid $NaBH_4$ and the resulting suspension was allowed to stir for 3 h. The reaction mixture was quenched with 5% $KHSO_4$ and extracted into ethyl acetate. The combined organic extracts were washed with brine and dried over $MgSO_4$. Flash chromatography (elution with 4% ether in methylene chloride) gave 81 mg of the alcohol (48) as a white solid. ^1H NMR consistent with structure.

(S)-Boc-Pipecolyl-(R and S)-5-(3-indolyl)-1-phenyl-2-pentyl ester (49)

The ester (49) was prepared from 80 mg (0.29 mmol) of alcohol (48), 82 mg (0.36 mmol) of (S)-Boc-pipecolic acid, 66 mg (0.34 mmol) of EDC and a catalytic amount of 4-pyrrolidinopyridine in 2.0 mL of CH_2Cl_2 as described above for the synthesis of (29) in Example 1. Flash chromatography (elution with 4:10:26 ether:methylene chloride:hexane) gave 108 mg of the diastereomeric ester (49) as a white foam. ^1H NMR consistent with structure.

(R and S)-5-(3-indolyl)-1-phenyl-2-pentyl (S)-pipecolate hydrochloride salt (50)

Anhydrous HCl was bubbled into a solution of 103 mg (0.21 mmol) of the ester (49) in 10 mL of EtOAc at -20°C for 10 min and then the reaction mixture was purged with

N_2. Concentration gave 108 mg of the crude amine (50) as the hydrochloride salt. ^1H NMR consistent with structure.

(R and S)-5-(3-indolyl)-1-phenyl-2-pentyl (S)-N-(3,4,5-trimethoxyphenylglyoxyl)pipecolate (11)

To a slurry of 108 mg of the crude amine hydrochloride (50) in CH_3CN at room temperature was added 91 μL (0.52 mmol) of N,N-diisopropylethylamine, 76 mg (0.31 mmol) of acid (31), and 111 mg (0.25 mmol) of the BOP reagent and the resulting mixture was stirred at room temperature for two days and was then concentrated to dryness. The residue was reconstituted into 75 mL of ethyl acetate and then sequentially washed with water, 5% $KHSO_4$, saturated $NaHCO_4$ and brine and then dried over $MgSO_4$ and concentrated. Flash chromatography (elution with 4% ether in methylene chloride) gave 56.7 mg of the diastereomeric amide (11) as a rotameric mixture. ^1H NMR (500 MHz, $CDCl_3$)δ 7.98 (d), 7.56 (t), 7.38-6.73 (m), 5.38-5.14 (m), 3.90 (m), 3.38 (brt), 3.10 (brt), 2.97-2.60 (m), 2.31 (d), 2.10 (d), 1.98-1.17 (m), 0.8 (m). R_f 0.51 (10% ether in methylene chloride).

EXAMPLE 6

Synthesis of (R and S)-2-Benzyl-4-phenyl-1-butyl (S)-N-(3,4,5-trimethoxyphenylglyoxyl)pipecolate (16)

(R and S)-2-Benzyl-4-phenyl-1-butyric acid (51)

To a solution of 1.06 g (6.43 mmol) of 4-phenylbutyric acid in 20 mL of THF at 0°C was added 193 mg (6.43 mmol) of solid NaH (80% in mineral oil). After

stirring at 0°C for 0.5 h, 3.2 mL (6.43 mmol) of lithium diisopropyl amide-THF complex (2.0 M) was added and the resulting red solution was stirred at 0°C for 45 min. To this mixture was added 765 μL (6.43 mmol) of benzylbromide and the solution was then allowed to stir overnight at room temperature. The reaction mixture was quenched by the slow addition of saturated $NaHCO_3$ and then washed with ether. The basic extracts were acidified with solid $KHSO_4$ and partitioned with ethyl acetate. The combined organic extracts were washed with brine, dried over $MgSO_4$ and concentrated to give 484 mg of the acid (51). ^1H NMR consistent with structure.

(R and S)-2-Benzyl-4-phenyl-1-butanol (52)

To a solution of 469 mg (1.84 mmol) of acid (51) in 3.0 mL of THF at -78°C was added 2.03 mL (2.3 mmol) of lithium aluminum hydride (1.0 M in THF) and the resulting solution was allowed to warm to room temperature and stirred overnight. The reaction mixture was quenched by the slow addition of Rochelle's salt and partitioned with ether. The combined ether extracts were washed with water and brine and dried over $MgSO_4$ and concentrated. Flash chromatography (elution with 2% ether in methylene chloride) to afford 264 mg of the alcohol (52). ^1H NMR consistent with structure.

(S)-Boc-Pipecolyl-(R and S)-2-Benzyl-4-phenyl-1-butyl ester (53)

The ester (53) was prepared from 264 mg (1.10 mmol) of alcohol (52), 302 mg (1.32 mmol) of (S)-Boc-pipecolic acid, 253 mg (1.32 mmol) of EDC and a catalytic amount of 4-pyrrolidinopyridine in 2.0 mL of CH_2Cl_2 as described

above for the synthesis of (29) in Example 1. Flash chromatography (elution with 1:5:14 ether:methylene chloride:hexane) gave 375 mg of the diastereomeric ester (53). ^{1}H NMR consistent with structure.

(R and S)-2-Benzyl-4-phenyl-1-butyl (S)-pipecolate hydrochloride salt (54)

Anhydrous HCl was bubbled into a solution of 375 mg (0.83 mmol) of the ester (53) in 10 mL of EtOAc at -20°C for 10 min and then the reaction mixture was purged with N_2. Concentration gave 352 mg of the crude amine (54) as the hydrochloride salt. ^{1}H NMR consistent with structure.

(R and S)-2-Benzyl-4-phenyl-1-butyl (S)-N-(3,4,5-trimethoxyphenylglyoxyl)pipecolate (16)

To a slurry of 54 mg (0.14 mmol) of the crude amine hydrochloride (54) in 2.0 ml of CH_3CN at room temperature was added 60 μL (0.35 mmol) of N,N-diisopropylethylamine, 50 mg (0.21 mmol) of acid (31), and 73 mg (0.16 mmol) of the BOP reagent and the resulting mixture was stirred overnight at room temperature and was then concentrated to dryness. The residue was reconstituted into 75 mL of ethyl acetate and then sequentially washed with water, 5% $KHSO_4$, saturated $NaHCO_4$ and brine and then dried over $MgSO_4$ and concentrated. Flash chromatography (elution with 4% ether in methylene chloride) gave 52.7 mg of the diastereomeric amide (16) as a rotameric mixture. ^{1}H NMR (500 MHz, $CDCl_3$)δ 7.21-7.01 (m), 5.41 (brs), 4.21 (dd), 4.08 (dd), 4.12 (d), 3.88 (d), 3.95 (s), 3.91 (s), 3.49 (d), 3.39 (dt), 2.80-2.62 (m), 2.38 (brt), 2.09 (brs), 1.87-1.20 (m). R_f 0.9 (1:3:26 methanol:ether:methylene chloride).

EXAMPLE 7

Synthesis of (R and S)-1-Phenyl-7-(2-pyridyl)-4-heptyl (S)-N-(tert-butylglyoxyl)pipecolate (21)

(E and Z)-3-(1,3-Dioxan-2-yl)-1-(2-pyridyl)-1-propene (55) and (56)

To a suspension of 4.6 g (10.2 mmol) of [2-(1,3-dioxan-2-yl)ethyl]triphenylphosphonium bromide (Aldrich Chemical Co.) in 50 mL of THF at 0°C was added 6.4 mL (10.2 mmol) of butyllithium (1.6 M in hexanes) and the resulting red solution was allowed to stir at 0°C for 0.5 h. To this solution was added 880 μL (9.3 mmol) of 2-pyridinecarboxaldehyde (Aldrich Chemical Co.) and the reaction mixture was allowed to stir at room temperature for 1 h and was then poured into water and partitioned with ether. The combined ether extracts were dried over $MgSO_4$ and concentrated. Flash chromatography (elution with 3:1 hexane:ethyl acetate) gave 0.43 g of E-3-(1,3-dioxan-2-yl)-1-(2-pyridyl)-1-propene (55) and 1.12 g of Z-3-(1,3-dioxan-2-yl)-1-(2-pyridyl)-1-propene (56). ^1H NMR consistent with structures.

1-(1,3-Dioxan-2-yl)-3-(2-pyridyl)propane (57)

Through a solution of 800 mg (4.2 mmol) of olefin (56) and 100 mg of 10% palladium on carbon was bubbled in a steady stream of hydrogen gas for a period of 10 min. The reaction mixture was then filtered through celite and concentrated to give 805 mg of the acetal (57) as a colorless oil. ^1H NMR consistent with structure.

4-(2-Pyridyl)-butyraldehyde (58)

A solution of 420 mg (2.2 mmol) of acetal (57) in 4.0 mL of THF and 3.0 mL of 4N HCl was stirred at room temperature for 1.5 h and was then neutralized by the slow addition of solid $NaHCO_3$. The reaction mixture was extracted with ethyl acetate, dried over $MgSO_4$ and concentrated to yield 288 mg of the aldehyde (58). ^1H NMR consistent with structure.

(R and S)-1-Phenyl-7-(2-pyridyl)-4-heptanol (59)

The alcohol (59) was prepared from 288 mg (1.93 mmol) of aldehyde (58) and 2.3 mL (2.3 mmol) of (27) in 3.0 mL of THF as described above for the synthesis of (28) in Example 1 to give 520 mg of the crude alcohol (59). ^1H NMR consistent with structure.

(S)-Boc-Pipecolyl-(R and S)-1-phenyl-7-(2-pyridyl)-4-heptyl ester (60)

The ester (60) was prepared from 520 mg (1.93 mmol) of alcohol (59), 442 mg (1.93 mmol) of (S)-Boc-pipecolic acid, 370 mg (1.93 mmol) of EDC and a catalytic amount of DMAP in 4.0 mL of CH_2Cl_2 and 4.0 mL of DMF as described above for the synthesis of (29) in Example 1. Flash chromatography (elution with 3:1 hexane:ethyl acetate) gave 740 mg of the diastereomeric ester (60) as an oil. ^1H NMR consistent with structure.

(R and S)-1-Phenyl-7-(2-pyridyl)-4-heptyl (S)-pipecolate (61)

The amine (61) was synthesized by treating 740 mg (1.54 mmol) of the ester (60) with 2.0 mL of trifluoroacetic acid in 5.0 mL of CH_2Cl_2 as described

above for the preparation of (30) in Example 1 giving 580 mg of the diastereomeric amine (61) as an oil. ^{1}H NMR consistent with structure.

(R and S)-1-Phenyl-7-(2-pyridyl)-4-heptyl (S)-N-methyloxalylpipecolate (62)

To a solution of 48 mg (0.13 mmol) of the amine (61) in 1.0 mL of CH_2Cl_2 at 0°C was added 33 μL (90.19 mmol) of N,N-diisopropylethylamine and 14 μL (0.15 mmol) of methyloxalyl chloride and the resulting solution was warmed to room temperature and allowed to stir overnight. The reaction mixture was diluted with ethyl acetate, washed with saturated NH_4Cl and brine, dried over $MgSO_4$ and then concentrated. Flash chromatography (elution with 25-30% ethyl acetate in hexane) gave 49 mg of the diastereomeric amide (62) as a mixture of rotamers. ^{1}H NMR consistent with structure.

(R and S)-1-Phenyl-7-(2-pyridyl)-4-heptyl (S)-N-(tert-butylglyoxyl)-pipecolate (21)

To a solution of the amide (62) in 1.2 mL of THF at -78°C was added tert-butyl lithium dropwise until TLC showed the consumption of the starting material. The reaction mixture was quenched with saturated NH_4Cl and partitioned with ethyl acetate. The combined organic extracts were washed with brine, dried over $MgSO_4$ and concentrated. Flash chromatography (elution with 30% ethyl acetate in hexane) gave the diasteromeric amide (21) as a mixture of rotamers. ^{1}H NMR (500 MHz, $CDCl_3$)δ 8.50 (t), 7.57 (t), 7.20-7.05 (m), 5.23 (d), 5.18 (d), 4.56 (d), 4.44 (br d), 4.13 (d), 3.69 (br d), 3.37-3.28 (m), 3.13-3.00 (m), 2.85-2.70 (m), 2.65-2.54 (m),

2.38-2.15 (m), 1.82-1.65 (m), 1.56-1.44 (m), 1.55-1.30 (m), 1.27 (s), 1.21 (s).

EXAMPLE 8

Synthesis of (R and S)-1-Phenyl-7-(3-pyridyl)-4-heptyl (S)-N-(3,4,5-trimethoxyphenylglyoxyl)pipecolate N-oxide (22)

(E and Z)-3-(1,3-Dioxan-2-yl)-1-(3-pyridyl)-propene (63)

To a suspension of 9.9 g (22.4 mmol) of [2-(1,3-dioxan-2-yl)ethyl]triphenylphosphoniumbromide (Aldrich Chemical Co.) in 50 mL of THF at 0°C was added 14.0 mL (22.4 mmol) of butyl lithium (1.6 M in hexanes) and the resulting red solution was allowed to stir at 0°C for 0.5 h. To this solution was added 1.8 mL (18.7 mmol) of 3-pyridinecarboxaldehyde (Aldrich Chemical Co.) and the reaction mixture was allowed to stir at room temperature for 1.5 h and was then poured into water and partitioned with ether. The combined ether extracts were dried over $MgSO_4$ and concentrated. Flash chromatography (elution with 2:1 hexane:ethyl acetate) gave 3.3 g of the alkene (63) as a mixture of olefin isomers. ^1H NMR consistent with structure.

1-(1,3-Dioxan-2-yl)-3-(3-pyridyl)propane (64)

Through a solution of 3.2 g (16.7 mmol) of olefin (63) and 300 mg of 10% palladium on carbon was bubbled a steady stream of hydrogen gas for a period of 10 min. The reaction mixture was then filtered through celite and concentrated to give 2.8 g of the acetal (64) as a colorless oil. ^1H NMR consistent with structure.

4-(3-Pyridyl)-1-butyraldehyde (65)

A solution of 1.5 g (7.8 mmol) of acetal (64) in 10.0 mL of THF and 10.0 mL of 4N HCl was stirred overnight at room temperature and was then neutralized by the slow addition of solid $NaHCO_3$. The reaction mixture was extracted with ethyl acetate, dried over $MgSO_4$ and concentrated to yield 1.1 g of the aldehyde (65). ^1H NMR consistent with structure.

(R and S)-1-Phenyl-7-(3-pyridyl)-4-heptanol (66)

The alcohol (66) was prepared from 1.1 g (7.4 mmol) of aldehyde (65) and 8.1 mL (8.1 mmol) of (27) in 30.0 mL of THF as described above for the synthesis of (28) in Example 1 to give 1.9 g of the crude alcohol (66). ^1H NMR consistent with structure.

(S)-Boc-Pipecolyl-(R and S)-1-Phenyl-7-(3-pyridyl)-4-heptyl ester (67)

The ester (67) was prepared from 1.65 g (6.12 mmol) of alcohol (66), 1.54 g (6.73 mmol) of (S)-Boc-pipecolic acid, 1.29 g (6.73 mmol) of EDC and a catalytic amount of DMAP in 8.0 mL of CH_2Cl_2 and 8.0 mL of DMF as described above for the synthesis of (29) in Example 1. Flash chromatography (elution with 2:1 hexane:ethyl acetate) gave 1.42 g of the diastereomeric ester (67) as an oil. ^1H NMR consistent with structure.

(R and S)-1-Phenyl-7-(3-pyridyl)-4-heptyl (S)-pipecolate (68)

The amine (68) was synthesized by treating 1.42 g (2.95 mmol) of the ester (67) with 2.0 mL of trifluoroacetic acid in 8.0 mL of CH_2Cl_2 as described

above for the preparation of (30) in Example 1 giving 1.02 g of the diastereomeric amine (68) as an oil. ^1H NMR consistent with structure.

(R and S)-1-Phenyl-7-(3-pyridyl)-4-heptyl (S)-N-(3,4,5-trimethoxyphenylglyoxyl)pipecolate (9)

The ester (9) was prepared from 995 mg (2.61 mmol) of the amine (68), 645 mg (2.87 mmol) of the acid (31) and 551 mg (2.87 mmol) of EDC in 6.0 mL of CH_2Cl_2 as described above in the synthesis of ester (3) in Example 1. Flash chromatography (elution with 3:1 acetone:hexane) gave 976 mg of the diasteromeric amide (9) as a mixture of rotamers. ^1H NMR consistent with structure.

(R and S)-1-Phenyl-7-(3-pyridyl)-4-heptyl (S)-N-(3,4,5-trimethoxyphenylglyoxyl)pipecolate N-oxide (22)

To a solution of 15 mg (0.02 mmol) of the amide (9) in 2.0 mL of CH_2Cl_2 at room temperature was added 9.3 μL (0.03 mmol) of 55% 3-chloroperoxybenzoic acid and the resulting solution was allowed to stir overnight at room temperature. Flash chromatography (elution with 100% acetone) gave 12.6 mg of the N-oxide (22) as a mixture of rotamers. ^1H NMR (500 MHz, $CDCl_3$)δ 8.10(m), 7.46-7.02(m), 5.88(d), 5.80(d), 5.06-5.00(m), 4.95-4.89(m), 4.61(m), 4.31(dd), 3.87(s), 3.84(s), 3.83(s), 3.81(s), 3.78(s), 3.50(br d), 3.27(ddd), 3.12(ddd), 3.00(ddd), 2.67-2.49(m), 2.32(br d), 1.86-1.78(m), 1.55-1.50(m), 1.39-1.22(m).

EXAMPLE 9

Synthesis of (R and S)-1-Phenyl-7-purinyl-4-heptyl (S)-N-(3,4,5-trimethoxyphenylglyoxyl)pipecolate (25)

4-Chlorobutyraldehyde (69)

To a solution of 19.1 g (0.15 mmol) of 4-chloro-1-butanol (Aldrich Chemical Co.) in 50 mL of CH_2Cl_2 at 0°C was added 1.0 g of powedered 4A molecular sieves and 38.7 g (0.18 mmol) of pyridinium dichromate and the resulting suspension was stirred at 0°C for 45 min. The reaction mixture was diluted with ether, filtered through celite and concentrated. The residue was vacuum distilled (bp 45-55°C) to 5.0 g of the aldehyde (69) as an oil. ^1H NMR consistent with structure.

(R and S)-1-Chloro-7-phenyl-4-heptanol (70)

The alcohol (70) was prepared from 182 mg (1.7 mmol) of aldehyde (69) and 1.9 mL (1.9 mmol) of (27) in 20.0 mL of THF as described above for the synthesis of (28) in Example 1 to give 128 mg of the crude alcohol (70). ^1H NMR consistent with structure.

(S)-Boc-Pipecolyl-(R and S)-1-Chloro-7-phenyl-4-heptyl ester (71)

The ester (71) was prepared from 128 mg (0.56 mmol) of alcohol (70), 156 mg (0.68 mmol) of (S)-Boc-pipecolic acid, 130 mg (0.68 mmol) of EDC and a catalytic amount of 4-pyrrolidinopyridine in 2.0 mL of CH_2Cl_2 as described above for the synthesis of (29) in Example 1. Flash

chromatography (elution with 1:5:14 ether:methylene chloride:hexane) gave 159 mg of the diastereomeric ester (71). ^{1}H NMR consistent with structure.

(S)-Boc-Pipecolyl-(R and S)-1-Phenyl-7-purinyl-4-heptyl ester (72)

To a solution of 34 mg (0.28 mmol) of purine in 3.0 mL of DMF at room temperature was added 8.4 mg (0.28 mmol) of solid NaH (80% in mineral oil) and the resulting solution was allowed to stir at room temperature for 10 min. To this reaction mixture was added 62 mg (0.14 mmol) of the chloride (71) and 10 mg of NaI and this mixture was stirred overnight at room temperature and then concentrated to dryness. The residue was dissolved into ethyl acetate, washed sequentially with water, saturated $NaHCO_3$, and brine and then dried over $MgSO_4$ and concentrated. Flash chromatography (elution with 15% 5:10:85 NH_4OH:MeOH:CH_2Cl_2 in CH_2Cl_2) gave 56 mg of the substituted purine (72) as an oil. ^{1}H NMR consistent with structure.

(R and S)-1-Phenyl-7-purinyl-4-heptyl (S)-pipecolate hydrochloride salt (73)

Anhydrous HCl was bubbled into a solution of 53.7 mg (0.10 mmol) of the ester (72) in 10 mL of EtOAc at -20°C for 10 min and then the reaction mixture was degassed with N_2. Concentration gave the crude amine (73) as the hydrochloride salt. ^{1}H NMR consistent with structure.

(R and S)-1-Phenyl-7-purinyl-4-heptyl (S)-N-(3,4,5-trimethoxyphenylglyoxyl)pipecolate (25)

To a slurry of the crude amine hydrochloride (73) in CH_3CN at room temperature was added 45 μL (0.26 mmol) of N,N-diisopropylethylamine, 37 mg (0.15 mmol) of acid (31), and 54 mg (0.12 mmol) of the BOP reagent and the resulting mixture was stirred at room temperature for two days and then was concentrated to dryness. The residue was reconstituted into 75 mL of ethyl acetate and then sequentially washed with water, 5% $KHSO_4$, saturated $NaHCO_4$ and brine and then dried over $MgSO_4$ and concentrated. Flash chromatography (elution with 1:4:36 MeOH:Et_2O:CH_2Cl) gave 26.5 mg of the diastereomeric amide (25) as a rotameric mixture. ^1H NMR (500 MHz, $CDCl_3$)δ 9.11 (s), 8.95 (m), 8.09 (m), 7.36-7.05 (m), 5.31 (m), 4.28 (m), 3.90 (m), 3.46 (br t), 3.20 (m), 2.58 (m), 2.28 (br d), 2.17-1.18 (m). R_f 0.1 (30% ether in methylene chloride).

DISCUSSION OF ASSAYS

Cell Source and Culture

Fresh peripheral blood lymphocytes (PBLs) from LeukoPak cells or whole blood from random normal blood donors (tested HIV-negative and hepatitis negative) are isolated and separated by density centrifugation over Histopaque 1077 (Sigma Chemical Co., St. Louis, MO). The murine CTLL cytotoxic T cell line and the human Jurkat T cell line are from ATCC (CTLL-2 ATCC TIB214, JURKAT CLONE E6-1 ATCC TIB152). The human allogeneic B cell lines used for activation of the fresh PBLs are EBV-transformed lymphocytes from normal healthy adult donors with two completely different HLA haplotypes. All cell lines were

routinely tested for the presence of Mycoplasma contamination using the Gibco Mycotect test kit and are Mycoplasma-free. Culture medium consists of RPMI 1640 (Gibco, Grand Island, NY) containing penicillin (50 U/ml) and streptomycin (50 μg/ml), L-glutamine 2 mM, 2 - mercaptoethanol (5×10^{-5}), 10% heat-inactivated FCS and 10 mM HEPES.

Compound Solutions and Titrations

All chemical stocks were dissolved in DMSO. Titrations of compounds were made into the medium the individual assay was carried out in, i.e., complete RPMI or HB 104 for final diluted concentrations, using multiple three-fold dilutions from 1 μM or 10 μM stock solutions.

MTT Assay

The MTT assay is a colorimetric technique to determine the toxicity of the compounds on growing lymphoid and non-lymphoid cell lines based on reduction of the tetrazolium salt by intact mitochondria (Mossman, T., J. Immunol. Methods 65:55 (1983)). Cell viability in the presence or absence of different concentrations of test compounds in serum-free medium (HB 104, HANA Biologic, Inc.) was assessed using MTT (3-[4,5-dimethyl-thiazoyl-2-yl]2,5-diphenyl-tetrazolium bromide). At 4 h before the end of the 3-day toxicity assay culture period, 20 μl of MTT dye (5 mg/ml in pH 7.2 PBS) were added to each microtiter well. At the end of the incubation time, most of the culture media was carefully aspirated out of each well. Then 100 μl of acidified isopropyl alcohol (0.04 N HCl) was added to solubilize the dye and optical density is read at 570 nm

minus OD at 630 nm (Molecular Devices Thermomax plate reader and Softmax software program, Menlo Park, CA). Results were compared with mean OD in controls (medium with no drugs) and doses causing 50% toxicity (TC_{50}) were calculated.

Mitogenesis Assays ("PMA" and "OKT3")

The inhibitory effect of test compounds on the proliferation of human PBLs in response to mitogens (Waithe, W.K. and K. Hirschhorn, Handbook of Experimental Immunology, 3d Ed. Blackwell Scientific Publications, Oxford (1978); Mishell, B.B. and S.M. Shiigi, Selected Methods in Cellular Immunology W.H. Freeman and Co., San Francisco, CA (1980)) was assessed by stimulation of 5 x 10^4 cells with OKT3 (10^{-4} dilution final) or PMA (10ng/ml) plus ionomycin (250 ng/ml) in the presence or absence of different concentrations of test compounds and control drugs (CsA, FK506, Pagamycin) in final volume of 200 μl per well in 96 well round bottomed plates. After 48 h incubation (37°C, 5% CO_2), cells were pulsed with 1 μCi of ^{3}H-thymidine, harvested 24 h later with a Tom Tek cell harvester, and counted in LKB β-scintillation counter. Results (cpm) were compared with controls with medium alone, and concentrations causing 50% reduction in counts (IC_{50}) were calculated.

MLR Bioassays ("LB" and "JVM")

Antigen activated proliferation of PBLs in a primary mixed lymphocyte reaction was assessed in the presence or absence of different concentrations of tested compounds and control drugs. 5 x 10^4 fresh PBLs were stimulated with 5 x 10^3 of Mitomycin C treated-allogeneic

EBV-transformed β-lymphoglastoid cells, LB and JVM, in a final volume of 200 µl per well in 96-well round-bottomed plates (Mishell, B.B. and S.M. Shiigi, Selected Methods in Cellular Immunology W.H. Freeman and Co., San Francisco, CA (1980); Nelson, P.A. et al., Transplantation 50:286 (1990)). Cultures were pulsed on day 6, harvested 24 h later and counted as in previous section.

IL-2 Microassay ("CTLL")

To determine if test compounds inhibit the later T cell activation process of cytokine utilization, the proliferative response of the IL-2 dependent CTLL-20 murine T cell line (ATCC) was assessed (Gillis, S. et al., J. Immunology 120:2027 (1978)). CsA and FK506 inhibit the production of IL-2 by activated T cells, whereas Rapamycin interferes with the utilization of IL-2. Rapamycin thus inhibits IL-2 dependent proliferation of the CTLLs, and CsA and FK506 do not (Dumont, F.J. et al., J. Immunology 144:251 (1990)). 3 x 10^3 CTLLs were exposed to different concentrations of test compounds and control drugs in the presence of 1 U/ml of human recombinant IL-2 (Genzyme, rIL-2) for 24 h. Four h after adding drugs, cells were pulsed with 1 µCI of 3H-thymidine, incubated for an additional 20 h (37°C, 5% CO_2), and then harvested and counted as previously described.

Bioavailability:

Bioavailability of compound (20) was determined in rats. A single dose of 50 mg/kg was administered by oral gavage or intraperitoneal (IP) injection in a vehicle

consisting of olive oil-10% ethanol. Thereafter, animals were sacrificed at 0.5, 1, 2, 4, 8 and 12 hr, blood was collected into sodium heparin and immediately frozen. Whole blood was extracted with acetonitrile-methanol (90/10 vol:vol) and the concentration of compound per ml of blood was determined by HPLC. Data indicate that IP administration yielded blood levels of 0.7μM, 22μM, 225μM, 45μM and 1μM at 0.5h, 1h, 2h, 4h, and 8h, respectively. After oral administration, blood levels of 0.5μM, 1μM, 2μM, 12μM, and 3μM were measured at .05h, 1h, 2h, 4h, and 8h, respectively.

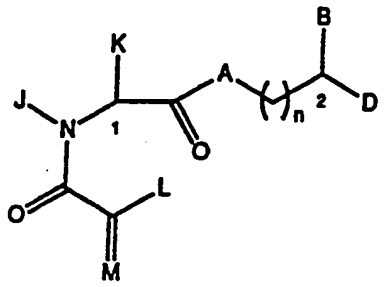
The blood levels attained after IP administration indicate adsorption from the peritoneal cavity into the circulation with maintenance of the bioactive structure. Blood levels achieved are sufficient for induction of immunosuppression.

Equivalents

Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims:

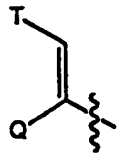
CLAIMS

1. A compound having immunosuppressive activity, represented by the formula:



and pharmaceutically acceptable salts thereof, wherein A is O, NH, or N-(C1-C4 alkyl);

wherein B and D are independently Ar, (C5-C7)-cycloalkyl substituted (C1-C6)-straight or branched alkyl or alkenyl, (C5-C7)-cycloalkenyl substituted (C1-C6)-straight or branched alkyl or alkenyl, or Ar substituted (C1-C6)-straight or branched alkyl, or alkenyl, wherein in each case, one or two carbon atoms of the straight or branched alkyl or alkenyl may be substituted with 1-2 heteroatoms selected from the group consisting of oxygen, sulfur, SO and SO_2, or



wherein Q is hydrogen, (C1-C6)-straight or branched alkyl or (C1-C6)-straight or branched alkenyl;

wherein T is Ar or substituted 5-7 membered cycloalkyl with substituents at positions 3 and 4 which are independently selected from the group consisting of hydrogen, oxo, hydroxyl, O-(C1-C4)-alkyl and O-(C1-C4)-alkenyl;

wherein Ar is selected from the group consisting of phenyl, 1-naphthyl, 2-naphthyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, monocyclic and bicyclic heterocyclic ring systems with individual ring sizes being 5 or 6 which may contain in either or both rings a total of 1-4 heteroatoms independently selected from oxygen, nitrogen and sulfur; wherein Ar may contain one to three substituents which are independently selected from the group consisting of hydrogen, halogen, hydroxymethyl, hydroxyl, nitro, trifluoromethyl, trifluoromethoxy, (C1-C6)-straight or branched alkyl, (C1-C6)-straight or branched alkenyl, O-(C1-C4)-straight or branched alkyl, O-(C2-C4)-straight or branched alkenyl, O-benzyl, O-phenyl, 1,2-methylenedioxy, amino, carboxyl and phenyl;

wherein L is either hydrogen or U; M is either oxygen or CH-U, provided that if L is hydrogen, then M is CH-U or if M is oxygen then L is U;

wherein U is hydrogen, O-(C1-C4)-straight or branched alkyl, O-(C1-C4)-straight or branched alkenyl, (C1-C6)-straight or branched alkyl, (C1-C6)-straight or branched alkenyl, (C5-C7)-cycloalkyl, (C5-C7)-cycloalkenyl substituted with (C1-C4)-straight or branched alkyl or (C2-C4)-straight or branched alkenyl, [(C1-C4)-alkyl

or (C2-C4)-alkenyl]-Ar or Ar (Ar as described above);

wherein J is hydrogen or C1 or C2 alkyl or benzyl; K is (C1-C4)-straight or branched alkyl, benzyl or cyclohexylmethyl; or wherein J and K may be taken together to form a 5-7 membered heterocyclic ring which may contain an O, S, SO or SO_2 substituent therein; and

wherein n is 0-3; and

wherein the stereochemistry at carbon positions 1 and 2 are independently (R) or (S).

2. An immunosuppressant compound of Claim 1, having an affinity for FK-506 binding protein.

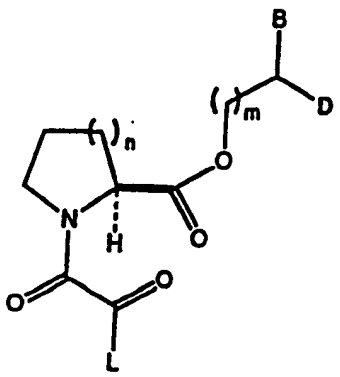
3. An immunosuppressant compound of Claim 1, capable of inhibiting the prolyl peptidyl cis-trans isomerase activity of the FK-506 binding protein.

4. An immunosuppressant compound of Claim 1, having a molecular weight below about 750 amu.

5. An immunosuppressant compound of Claim 1, having a molecular weight below about 500 amu.

6. An immunosuppressant compound of Claim 1, wherein the stereochemistry at carbon position 1 is S.

7. An immunosuppressant compound of Claim 1, wherein J and K are taken together and is represented by the formula:



wherein n is 1 or 2 and m is 0 or 1.

8. An immunosuppressant compound of Claim 7, wherein B is selected from the group consisting of 3-(2-pyridyl)propyl, 3-phenylpropyl, 2-phenoxyphenyl, phenyl, 2-(3-pyridyl)ethyl, E-3-[trans-(4-hydroxycyclohexyl)]-2-methyl-prop-2-enyl, 3-(3-pyridyl)propyl, benzyl, 2-phenylethyl, 2-(4-methoxyphenyl)ethyl, 3-(N-benzimidazoly)propyl, 3-(4-methoxyphenyl)propyl, 3-[N-(7-azaindolyl)propyl, 3-(N-purinyl)propyl, 3-(3-pyridyl)-N-oxide, 3-(4-hydroxymethylphenyl)propyl, 3-(2-thienyl)propyl, 3-(4-carboxyphenyl)propyl, 4-phenylbutyl, 2-hydroxymethylphenyl, 2-allyloxyphenyl, 3-(3-hydroxymethylphenyl)propyl, 3-(3-carboxyphenyl) propyl, 3-hydroxymethylphenyl, 2-hydroxyphenyl, 3-pyridyl and 5-phenylpentyl;

D is selected from the group consisting of 3-phenylpropyl, 2-phenoxyphenyl, 3-(3-indolyl)-propyl, 2-phenylethyl, 4-phenylbutyl and 3-(4-methoxyphenyl)propyl; and

L is selected from the group consisting of 3,4,5-trimethyloxyphenyl, phenyl, tert-butyl, 3-benzyloxyphenyl, 3-allyloxyphenyl and 3-isopropoxyphenyl.

9. A compound having immunosuppressive activity represented by any of the structures shown in Table 1 and having an affinity for FK-506 binding protein.

10. A composition for use in suppressing an immune response in a mammal, comprising an immunosuppressant compound of Claim 1 having an affinity for FK-506 binding protein and having a molecular weight below about 750 amu, in a physiologically acceptable vehicle.

11. Use of an immunosuppressant compound of Claim 1 having an affinity for FK-506 binding protein and having a molecular weight below about 750 amu in a physiologically acceptable vehicle; for the manufacture of a medicament for use in suppressing an immune response in a mammal.

12. A composition or use according to any one of Claims 10 and 11, wherein the immune response to be suppressed in an autoimmune response or an immune response associated with graft rejection.

13. A composition or use according to any one of Claims 10, 11 and 12, wherein the immunosuppressant compound is represented by the structures shown in Table 1.

14. A composition or use according to any one of Claims 10, 11, 12 and 13, further comprising an immunosuppressant selected from the group consisting of

cyclosporin, rapamycin, FK506, 15-deoxyspergualin, OKT3 and azathioprine.

15. A composition or use according to any one of Claims 10, 11, 12, 13 and 14, further comprising a steroid.

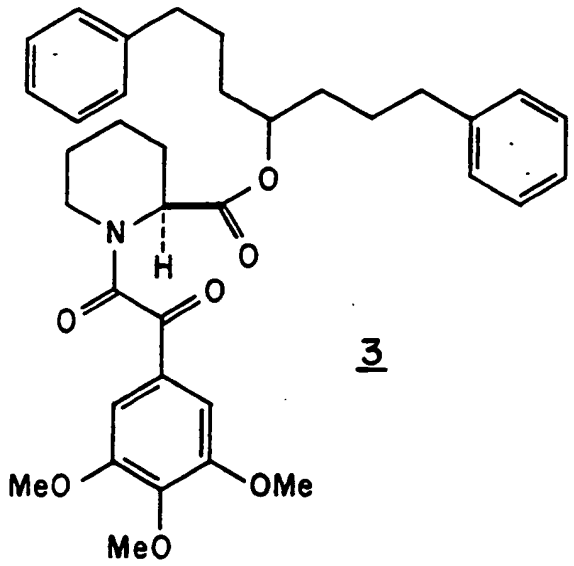


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FIG. IA

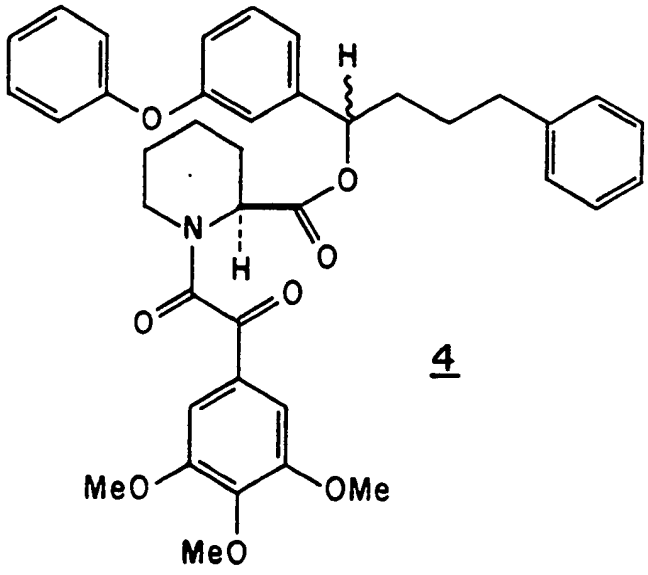

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FIG. IB

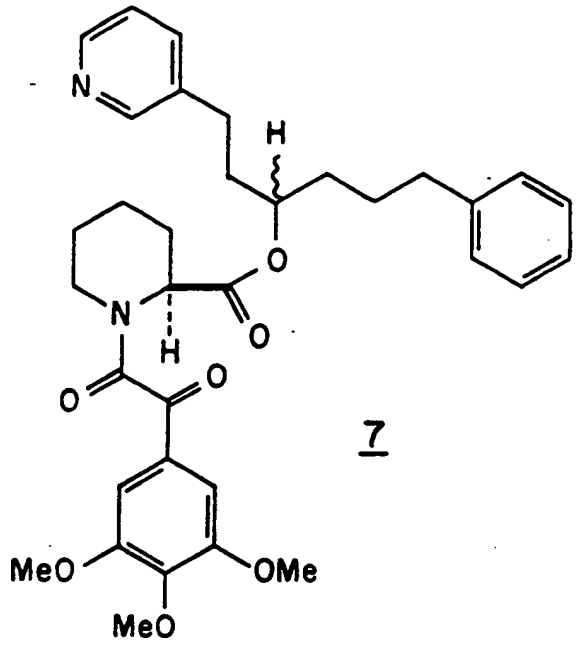

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FIG. IC

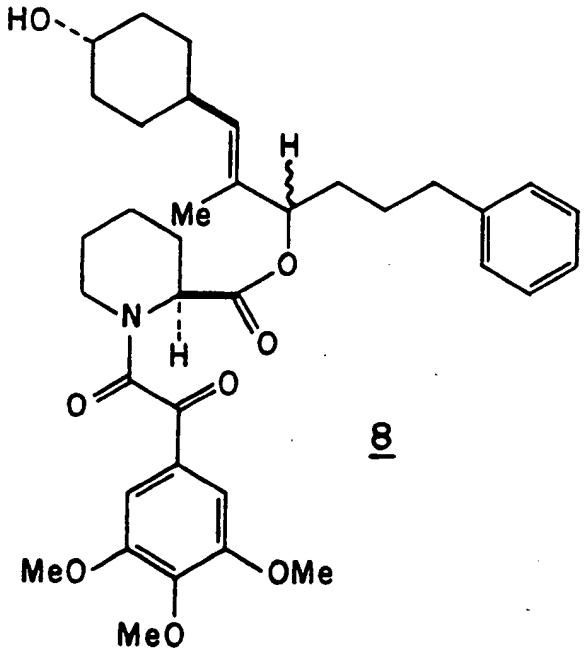

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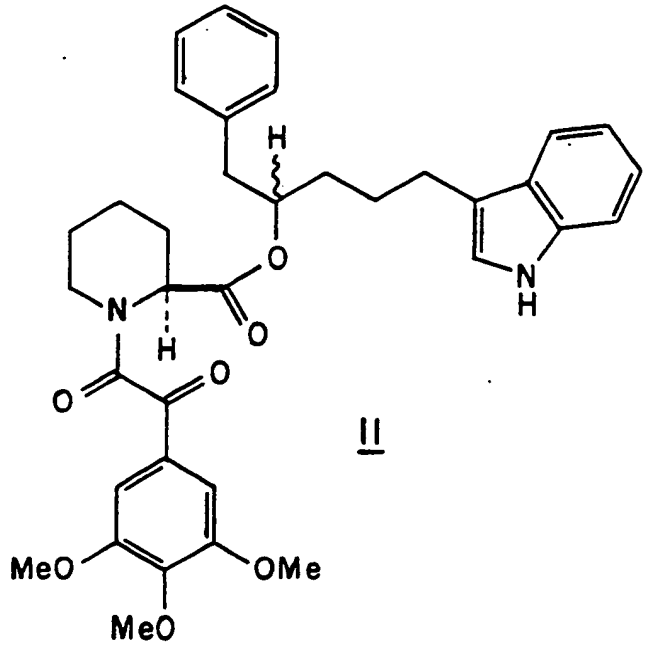
FIG. ID



II

FIG. 1E

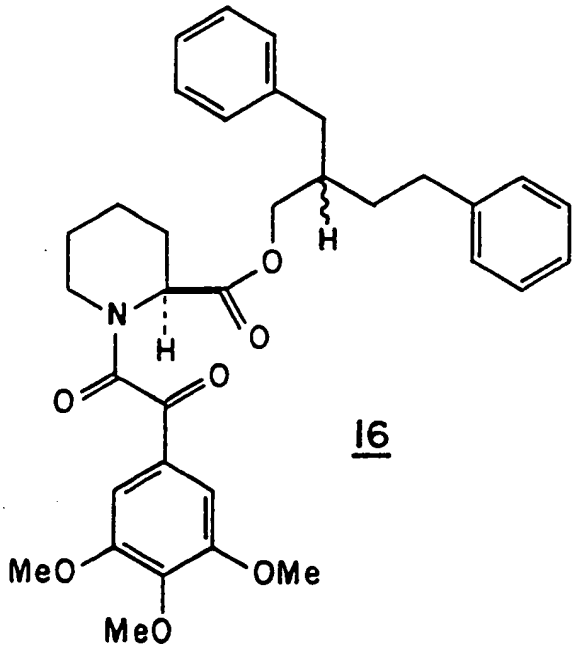


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FIG. 1F

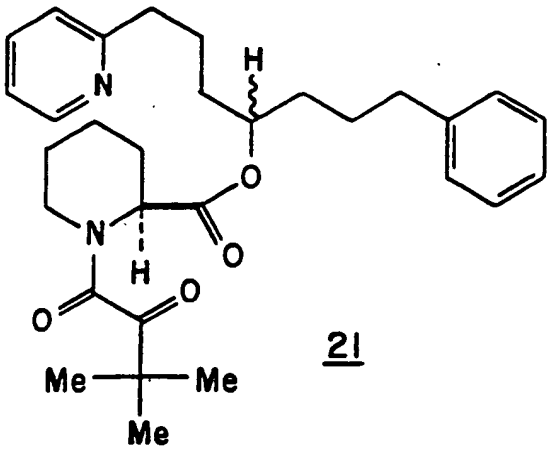

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FIG. 1G

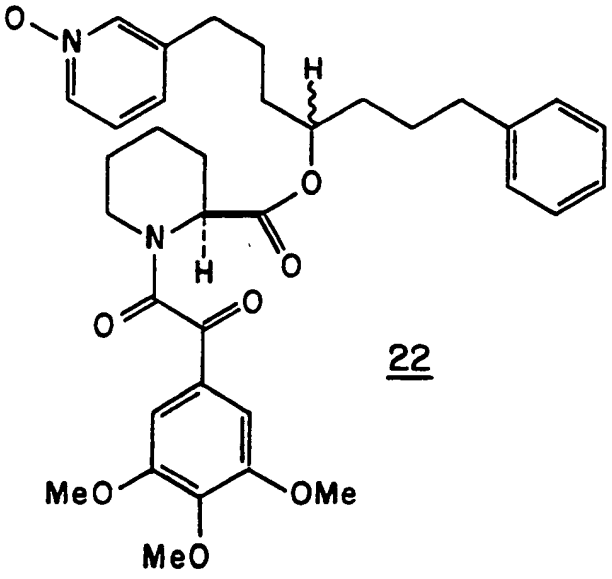

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FIG. 1H

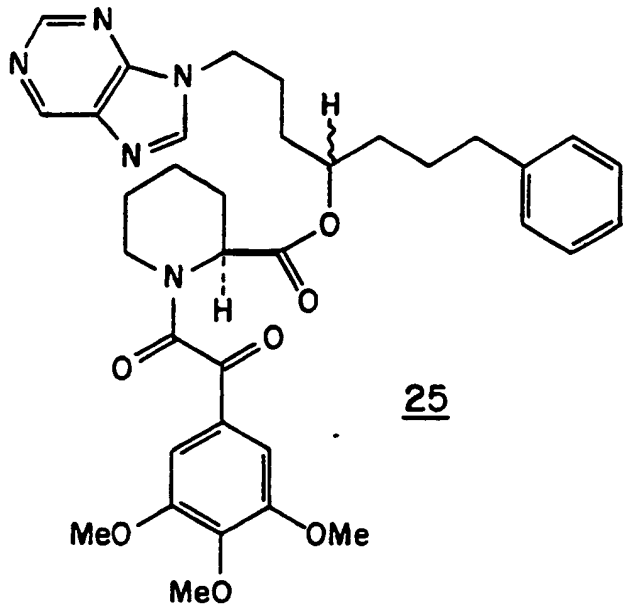

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FIG. 11

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C07D207/16; C07D211/60; C07D401/06; C07D409/06
C07C235/72; A61K31/395; A61K31/40; A61K31/215

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C07D ; C07C ; A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
P,,, A	WO,A,9 200 278 (VERTEX PHARMACEUTICALS INCORPORATED) 9 January 1992 - whole document -	7-10, 12-15
P,,, A	EP,A,0 457 163 (HOECHST AG) 21 November 1991 see examples 4,11; table 1	7-10, 12-15
A	EP,A,0 172 458 (SUNTORY LIMITED) 26 February 1986 - whole document -	7,8
A	EP,A,0 384 341 (KABUSHIKI KAISHA YAKULT HONSHA) 29 August 1990 - whole document -	7,8

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"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
15 SEPTEMBER 1992	05. 10. 92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	HERZ C.

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ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 9203913 SA 60567

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 15/09/92
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Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9200278	09-01-92	AU-A-	8285591	23-01-92
EP-A-0457163	21-11-91	DE-A-	4015255	14-11-91
		AU-A-	7622191	14-11-91
		CN-A-	1056683	04-12-91
EP-A-0172458	26-02-86	JP-A-	61037764	22-02-86
		AU-B-	575668	04-08-88
		AU-A-	4559785	06-02-86
		US-A-	4743616	10-05-88
EP-A-0384341	29-08-90	JP-A-	2218663	31-08-90

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